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Short Communication

Molecular Analysis of *Salmonella* Enteritidis Isolates Resistance to Ampicillin and Streptomycin from Three Outbreaks of Food Poisoning in Shiga Prefecture

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SUMMARY: In 1998 and 1999, there were three outbreaks caused by *Salmonella* Enteritidis in Shiga Prefecture. One outbreak was suspected to be a diffuse outbreak, caused by frozen cream puffs that had been sold in chain stores throughout Shiga Prefecture between the beginning of September and the beginning of October, 1998. The other outbreaks occurred in May and in August, 1999. All isolates of the three outbreaks showed an identical lysis pattern against the typing phage, though this pattern did not conform to the current scheme, so-called RDNC. In addition all isolates were resistant to ampicillin and streptomycin. However, the patterns of pulsed-field gel electrophoresis strongly indicated that the three outbreaks were actually independent.

Salmonella Enteritidis (SE) has been isolated most frequently among *Salmonella enterica* in Japan since 1989 (1). Consumption of contaminated egg products has been shown to be a risk factor both in outbreaks and in sporadic cases (1).

Serotyping, antimicrobial susceptibility tests, and bacteriophage typing have been employed to determine the epidemiological association of isolates from patients, or to trace possible routes of transmission from animals to humans (2-3). Recently, molecular methods such as pulsed-field gel electrophoresis (PFGE) have proved to be highly discriminatory. These epidemiological markers have made it possible to identify diffuse outbreaks (4-7).

Shiga Prefecture suffered from three outbreaks caused by SE in September 1998 and May and August 1999, respectively. We report here a comparative investigation among isolates of the three outbreaks and sporadic cases in the same period, and reveal that one outbreak was a diffuse outbreak, and the three were caused by independent SE strains, respectively.

One hundred forty-two isolates of SE were examined. Thirty were isolated from the three outbreaks, 20 from outbreak A, 3 from outbreak B, and 7 from outbreak C. One hundred twelve were from sporadic cases in Shiga Prefecture in 1998. The details of the three outbreaks are provided in Table 1. Patients of outbreak B were residents of Shiga Prefecture who had traveled to the Kyusyu district and were infected there. Outbreak C occurred at the hospital in Shiga Prefecture. All isolates were identified as SE by the API 20E system (BioMerieux S.A., Marcy l'Etoile, France), the Kauffman serotyping scheme (8), and commercially purchased antisera (Denka Seiken, Tokyo).

Bacteriophage typing was performed by the method of Ward et al. (9).

The antimicrobial susceptibility tests were carried out using the Senci-Disc (BBL Microbiology System, Cockeysville, Md., USA) and were performed by the Kirby-Bauer method (10). Disks containing ampicillin, streptomycin, kanamycin, gentamicin, tetracyclin, cefazolin, chloramphenicol, norfloxacin, sulfomethoxazole and trimethoprim, and fosfomycin were used.

Table 1. Characteristics of *S.* Enteritidis isolates resistace to ampicillin and streptomycin from outbreaks and sporadic patients

	Date of outbreak	Place of occurrence	Number of isolates	Source	Phage type
Outbreak A	Sep. /1998	Kindergarten	9	Patients' feces	RDNC
			11	Frozen cream puffs	RDNC
Outbreak B	May/1999	Restaurant	3	Patients' feces	RDNC
Outbreak C	Aug./1999	Hospital	7	Patients' feces	RDNC
Sporadic case	Sep.–Nov./1998		32	Patients' feces	RDNC
	Oct. /1998		1	Patient's feces	4

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Extraction of DNA and the conditions for PFGE were based on the method of Terajima et al. (3). Restriction endonuclease *Bln*I (Takara, Shiga) was used.

All 30 isolates from the three outbreaks were found to be resistant to ampicillin and streptomycin. The results of the bacteriophage typing for the isolates were identical, although the lysis patterns did not conform to any current scheme, so-called RDNC (they reacted but did not conform).

The PFGE patterns of isolates from each outbreak differed from each other, though those of isolates within each outbreak were indistinguishable or quite similar to one another (Fig. 1a). For example, although five of seven isolates from outbreak C showed identical PFGE patterns, the patterns of two isolates were different in two or three bands compared to the major pattern (Fig. 1a). These two isolates could be included in a part of outbreak C, according to the criteria of Tenovar et al. (11). Thus, the three outbreaks were indicated to be independent based on the PFGE results, despite the conflicting results of antimicrobial susceptibility tests and bacteriophage typing.

Thirty-three of 112 sporadic isolates were resistant to ampicillin and streptomycin, as were those of the three outbreaks, and these 33 were further examined by bacteriophage typing and PFGE. Thirty-two were identified as RDNC whose lysis patterns were identical to those of the three outbreaks. The other was identified as phage type (PT) 4. In the PFGE analysis, 26 of 32 sporadic RDNC isolates showed patterns identical to those of outbreak A; five and one showed patterns with a difference in one and four bands, respectively, compared to those of outbreak A; and the latter PT4 isolate showed patterns quite different from any of the others (Fig. 1b). The PFGE results suggest that the patterns of the 32 sporadic isolates could be included in the same subtype as that of outbreak A, according to the criteria of Tenovar et al. (11). Furthermore, most of the 32 isolates were isolated between the beginning of September and the beginning of December in 1998, overlapping the period of outbreak A (Fig. 2). As such, the period of the isolation of the sporadic SE and the sold period of contaminated frozen cream puffs partly overlapped. Thus, these sporadic cases might have been a part of outbreak A; that is, outbreak A can be considered a diffuse outbreak affecting the whole of Shiga Prefecture.

In combination with antimicrobial susceptibility tests, bacteriophage typing and PFGE appear to be of value in epidemiological investigations of SE (3,12). As for phage

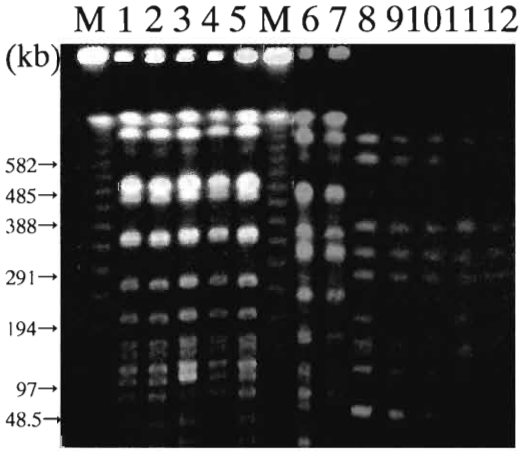

Fig. 1a. PFGE patterns of SE chromosomal DNA fragments digested with *Bln*I.
Lane M: DNA ladder, Lanes 1-2: isolates from frozen cream puffs, 3-5: isolates from patients (outbreak A), 6-7: isolates from outbreak B, 8-12: isolates from outbreak C.

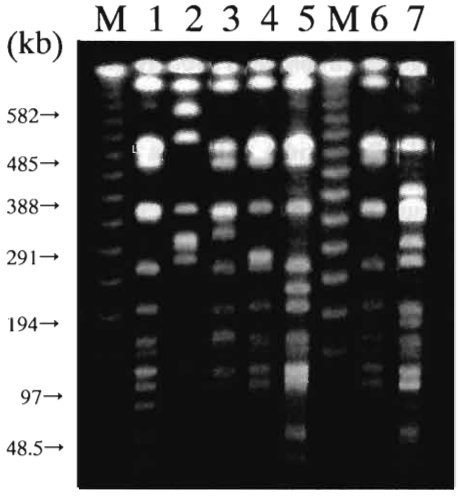

Fig. 1b. PFGE patterns of SE chromosomal DNA fragments digested with *Bln*I.
Lane M: DNA ladder, Lane 1: isolates from a frozen cream puff, 2: isolates from sporadic patient (PT4), 3-7: isolates from sporadic patients (RDNC).

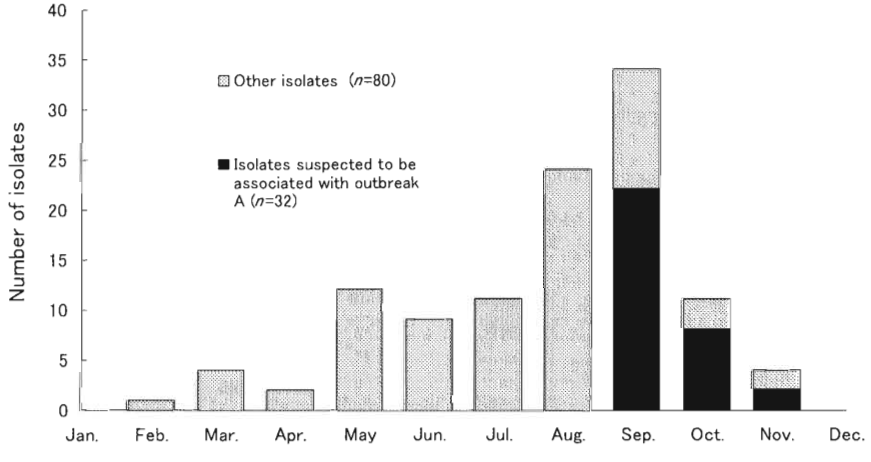

Fig. 2. Distribution of *S.* Enteritidis isolates from sporadic cases in 1998.

types, isolates with RDNC have shown a recent increase in Japan though PT1 and PT4 remain predominant (1), suggesting that SE strains with similar characteristics to those in this study are spreading (13). Coordination of field and molecular epidemiological investigations will be important in elucidating associations among both outbreaks and sporadic cases.

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